Novel Pneumococcal Serotypes 6C and 6D: Anomaly or Harbinger

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Clinical use of the 7-valent pneumococcal protein conjugate (PCV7) vaccine, which includes serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, dramatically reduced invasive pneumococcal disease (IPD); however, the effectiveness was diminished due to serotype shift. Although shift due to known serotypes was anticipated, shift by misidentified serotypes was unexpected. We describe the experience with newly recognized serotypes 6C and 6D, which were mistyped as serotypes 6A and 6B, respectively. Although serotype 6D caused only occasional infections, IPD due to serotype 6C disease expanded in the PCV7 era. Subsequent studies showed that PCV7 provided cross-protection against serotype 6A but not serotype 6C. The 13-valent pneumococcal protein conjugate (PCV13) vaccine, which includes PCV7 serotypes plus serotypes 1, 3, 5, 6A, 7F, 19A, may provide protection against IPD due to serotypes 6C and 6D. Regardless, this narrative illustrates the potential impact of unrecognized serotypes on the efficacy of a serotype-specific vaccine.

Given the propensity of Streptococcus pneumoniae to cause disease, the prevention of invasive pneumococcal disease (IPD) has been a major priority for several decades. Although S. pneumoniae includes approximately 90 serotypes [1], most pediatric and drug-resistant infections are due to a limited number of serotypes. Although the 23-valent pneumococcal polysaccharide vaccine (PPSV23) includes these serotypes, children aged <2 years cannot mount an immune response to this vaccine. Fortunately, the 7-valent pneumococcal conjugate vaccine (PCV7) has been remarkably successful at reducing the incidence of IPD in both children and adults through immunization and herd immunity, respectively [2]. As might be expected, serotype replacement with nonvaccine serotypes has occurred with the most common culprit, serotype 19A [3], resulting in significant albeit small increases in IPD in the United States [2, 4]. An unexpected outcome of the licensure of PCV7 is the increase in IPD due to newly discovered serotype 6C infections [5, 6]. Although the magnitude of serotype replacement due to this novel serotype has been relatively small, the implications of the discovery of both serotypes 6C and 6D are substantial [7, 8]—namely, that the efficacy of a pneumococcal conjugate vaccine can be eroded by hence undiscovered serotypes. In an effort to both clearly summarize recent findings and provide insight into the potential shortcomings of future serotype-specific vaccine prevention programs, the discovery and impact of serotypes 6C and 6D is described below.

SEROTYPES 6A AND 6B

Streptococcus pneumoniae serogroup 6, described in the 1920s, was thought to include only serotypes 6A and 6B [9]. Using the Pneumotest-Latex kit, which consists of 14 different pooled pneumococcus antisera (Staten Serum Institut), strains from serogroup 6 were classified by positive reactions in pools B and Q using the checkerboard method [10] (Table 1). To determine whether the isolate was serotype 6A or 6B, serotype-specific factor antiserum was mixed with a drop of the
culture and examined with a ×100 magnification oil immersion lens for the quellung reaction, or Neufeld test [11]. Historically, serotype 6A isolates were reactive with factor serum 6b, and serotype 6B isolates were reactive with factor serum 6c (Table 2).

In addition to the quellung reaction, a pyrosequencing assay of the wciP gene can usually discriminate between serotype 6A and 6B strains. A single-nucleotide polymorphism (SNP) at base-pair (bp) position 584 (residue 195) of the wciP gene is typically responsible for a difference in the rhamnose ribitol linkage associated with the production of serotypes 6A and 6B capsular polysaccharide (PS): a serine is associated with a 1, 3 linkage in the serotype 6A PS, and an asparagine is associated with a 1, 4 linkage in the serotype 6B PS [12] (Figure 1). The discovery of strains that produce both serotype 6A and 6B capsular polysaccharide (PS): a serine is associated with a 1, 4 linkage in the serotype 6B PS, and an asparagine is associated with a 1, 3 linkage in the serotype 6A PS, and an asparagine is associated with a 1, 4 linkage in the serotype 6B PS. The wciP gene is typically responsible for a difference in the rhamnose ribitol linkage associated with the production of serotypes 6A and 6B capsular polysaccharide: a serine is associated with a 1, 3 linkage in the serotype 6A PS, and an asparagine is associated with a 1, 4 linkage in the serotype 6B PS.

Table 1. Serogrouping Streptococcus pneumoniae Using Pneumotest-Latex Kit of 14 Different Pooled Antisera*

<table>
<thead>
<tr>
<th>Pool</th>
<th>P</th>
<th>Q</th>
<th>R</th>
<th>S</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>18</td>
<td>4</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>19</td>
<td>6</td>
<td>3</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>9</td>
<td>11</td>
<td></td>
<td></td>
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<tr>
<td>E</td>
<td>12</td>
<td>10</td>
<td>33</td>
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<tr>
<td>F</td>
<td>17</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>29</td>
<td>34</td>
<td>35</td>
<td>42</td>
<td>47</td>
</tr>
<tr>
<td>H</td>
<td>14</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>25</td>
<td>38</td>
<td>43</td>
<td>44</td>
<td>45</td>
</tr>
</tbody>
</table>

Adapted from Slotved et al [10].
* Commercially available from Staten Serum Institut.

Table 2. Revised Serologic and Molecular Properties of Serogroup 6 Isolates Using Modified Quellung Reagents and Polymerase Chain Reaction of wciN Gene

<table>
<thead>
<tr>
<th>Reaction Patterns With Known Serotypes</th>
<th>6A</th>
<th>6B</th>
<th>6C</th>
<th>6D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor sera*</td>
<td></td>
<td>6b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6c</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6d</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>wciNα gene</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>wciNβ gene</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

* Sera modified by Staten Serum Institut in January 2009.

only 50% sequence homology, with the serotype 6A wciN gene approximately 200 bp longer in length. The serotype 6A wciN gene is typically responsible for a difference in the rhamnose ribitol linkage associated with the production of serotypes 6A and 6B capsular polysaccharide: a serine is associated with a 1, 3 linkage in the serotype 6A PS, and an asparagine is associated with a 1, 4 linkage in the serotype 6B PS. The wciP gene is typically responsible for a difference in the rhamnose ribitol linkage associated with the production of serotypes 6A and 6B capsular polysaccharide: a serine is associated with a 1, 3 linkage in the serotype 6A PS, and an asparagine is associated with a 1, 4 linkage in the serotype 6B PS. The discovery of strains that produce both serotype 6A and 6B capsular polysaccharide (PS): a serine is associated with a 1, 3 linkage in the serotype 6A PS, and an asparagine is associated with a 1, 4 linkage in the serotype 6B PS. The wciP gene is typically responsible for a difference in the rhamnose ribitol linkage associated with the production of serotypes 6A and 6B capsular polysaccharide: a serine is associated with a 1, 3 linkage in the serotype 6A PS, and an asparagine is associated with a 1, 4 linkage in the serotype 6B PS.

**SEROLOGY 6C**

In 2007, isolates that were negative for pneumococcal antisera pools A through I, including pool B, were detected at an increased frequency. Interestingly, these isolates were positive for the Omni serum, pool Q, and group 6 antiserum, suggesting a serogroup 6 classification; however, pool B reaction was unexpectedly weak or negative. These strains, which were classified as “dodgy 6As” [15], were found to interact with only 1 of 2 serotype 6A monoclonal antibodies (mAbs) [16, 17]. These dodgy 6As were subsequently classified as serotype 6C based on their genetic sequence and capsular PS structure.

Whereas the serotype 6A and 6C capsular loci had highly related genetic sequences overall, the wciN capsular gene had only 50% sequence homology, with the serotype 6A wciN gene approximately 200 bp longer in length. The serotype 6A wciN capsular gene, also denoted as wciNα, was found to encode a galactosyl transferase; in contrast, the serotype 6C wciN capsular gene, also denoted as wciNβ, was found to encode a glucosyl transferase [7]. As might be expected, the serotype 6A capsule contained galactose, whereas the serotype 6C capsule contained glucose (Figure 1). [8].

**Identification of Serotype 6C Strains**

Using the inability of a strain to bind to 1 of the 2 serotype 6A mAbs as the gold standard method to detect a serotype 6C strain, serotype 6C-specific polymerase chain reaction (PCR) assays were developed [5, 18, 19]. If quellung-based serotyping is first used to differentiate serotype 6A/6C strains from 6B strains, 3 different published PCR-based assays can accurately identify serotype 6C strains among a mixture of serotype 6A and 6C strains [5, 18, 19]. Moreover, both the Statens Serum Institut and the Centers for Disease Control and Prevention have developed a modified factor antisera 6b* to replace factor antisera 6b, which is specific for serotype 6A and does not cross react with serotype 6C [20–22]. In addition, a novel antiserum specific for serotype 6C, factor antiserum 6d, has been developed and validated using PCR analysis [20] and sequence analysis [21] of the wciN gene.

**Evolution of Serotype 6C**

Serotype 6C strains have been detected worldwide (Table 3) and have been present in the United States since at least 1979 [7]. Two key discoveries indicate that the wciNβ gene from serotype 6C strains collected worldwide over a decade have > 0.1% sequence variation [23]. Second, all of the serotype 6C strains tested, unlike serotype 6A and serotype 6B strains, have a 6 nucleotide frameshift deletion in the wzy gene. Moreover, an evolutionary
Of the wcIN genes are indicated with variable density of dots; serotypes 6A and 6B carry the wcINα gene, and serotypes 6C and 6D carry the wcINβ gene. Likewise, the wcIP genes are indicated by the vertical and horizontal bars; typical serotype 6A and 6C strains have a wcIP gene with a serine at residue 195 (Ser195), whereas typical serotype 6B and 6D strains have an asparagine (Asp195) at this position. Adapted from Bratcher PE et al [23]. Chemical structure of serotypes 6A, 6B, 6C, and 6D: 6A: \( \rightarrow \)2)-Galactose – (1 → 3) – Glucose – (1 → 3) – Rhamnose – (1 → 3) – Ribitol – (5 → P 6B: \( \rightarrow \)2)-Galactose – (1 → 3) – Glucose – (1 → 3) – Rhamnose – (1 → 4) – Ribitol – (5 → P 6C: \( \rightarrow \)2)-Glucose – (1 → 3) – Glucose – (1 → 3) – Rhamnose – (1 → 3) – Ribitol – (5 → P 6D: \( \rightarrow \)2)-Glucose – (1 → 3) – Glucose – (1 → 3) – Rhamnose – (1 → 4) – Ribitol – (5 → P \( \rightarrow \))

**Figure 1.** Capsular operon of serotypes 6A, 6B, 6C, and 6D. The wcIN genes are indicated with variable density of dots; serotypes 6A and 6B carry the wcINα gene, and serotypes 6C and 6D carry the wcINβ gene. Likewise, the wcIP genes are indicated by the vertical and horizontal bars; typical serotype 6A and 6C strains have a wcIP gene with a serine at residue 195 (Ser195), whereas typical serotype 6B and 6D strains have an asparagine (Asp195) at this position. Adapted from Bratcher PE et al [23].

Chemical structure of serotypes 6A, 6B, 6C, and 6D:

- **6A:** \( \rightarrow \)2)-Galactose – (1 → 3) – Glucose – (1 → 3) – Rhamnose – (1 → 3) – Ribitol – (5 → P
- **6B:** \( \rightarrow \)2)-Galactose – (1 → 3) – Glucose – (1 → 3) – Rhamnose – (1 → 4) – Ribitol – (5 → P
- **6C:** \( \rightarrow \)2)-Glucose – (1 → 3) – Glucose – (1 → 3) – Rhamnose – (1 → 3) – Ribitol – (5 → P
- **6D:** \( \rightarrow \)2)-Glucose – (1 → 3) – Glucose – (1 → 3) – Rhamnose – (1 → 4) – Ribitol – (5 → P

Molecular Epidemiology of Serotype 6C
Serotype 6C strains share more multilocus sequence typing alleles with serotype 6A strains than either serotype 6B or 19A strains in the www.mlst.net database [23], providing indirect evidence that the serotype 6C capsular locus preferentially inserts into the serotype 6A background. The circulation of some of these serotype 6C clones can vary by population and location [24–27]. In Spain, two-thirds (142 of 213) of the serotype 6C strains causing IPD were related to a single clonal group (ST224-complex) [27]. In Southern Israel, 55.5% (27 of 49) of the serotype 6C strains isolated from the nasopharynx (NP) of Jewish children belonged to 1 clone (ST1692); in contrast, 88.1% (37 of 42) of the serotype 6C strains isolated from the NP of Bedouin children belonged to an unrelated clone (ST3531) \( (P < .01) \) [26]. In other studies, as serotype 6C emerged, the genetic diversity increased. For example, the number of serotype 6C clones increased from 2 to 7 over a 3-year period among children from Massachusetts who carried pneumococci in their NP [25]. Moreover, the penicillin nonsusceptible (pcn-NS) serotype 6C strains detected throughout the United States are genetically diverse, including variants of the North Carolina 6A-23 clone [24].

Antibiotic Susceptibility Patterns of Serotype 6C Strains
Serotype 6C strains have now been detected on 6 different continents from a variety of sources. The frequency of serotype 6C pcn-NS strains, defined as a penicillin minimum inhibitory concentration (MIC) \( \geq 0.12 \mu g/mL \), ranges 0%–77% (Table 1). Although serotype 6A is associated with penicillin nonsusceptibility more often than serotype 6C [5, 18, 19, 26–37] (Table 3), the frequency of pcn-NS serotype 6C isolates causing IPD is increasing in some countries. In the United States, penicillin nonsusceptibility has increased from 2% to 5% over the last 3 years [24, 36].
States, cases of IPD due to pcn-NS serotype 6C markedly increased from 11.4% (4 of 35) in 1999 to 31.2% (44 of 141) in 2007 [5]. In Portugal, the frequency of multidrug-resistant serotype 6C strains increased between 1996 and 2007, with all, 17.9% (19 of 106), of the multidrug resistant strains detected in 2006 and 2007 [34].

### EPIDEMIOLOGY OF SEROTYPE 6C IN THE PCV7 ERA

The epidemiologic data clearly indicate that serotype 6C disease has been increasing since the introduction of PCV7 in 2000. Using a population-based database of persons residing in 8 regions of the United States, the incidence of serotype 6C IPD was found to have markedly increased from 0.22 to 0.58 cases per 100 000 population between 1999 and 2007 [5]. These findings were echoed in a population-based study of Navajo and White Mountain Apache communities, with the incidence of serotype 6C IPD increasing from 0.3 to 1.5 cases per 100 000 population after the introduction of the PCV7 vaccine [6]. Likewise, IPD due to serotype 6C, as a proportion of infections due to serogroup 6, increased between 2000 and 2009 at 8 US children’s hospitals [38]. Thus, serotype 6C is now the most prevalent cause of serogroup 6 IPD in the United States [5]. Moreover, serotype 6C accounted for 5% (45 of 898) of IPD due to pcn-NS strains in 2007 [24]. In addition, the incidence of serotype 6C IPD is increasing in Australia, with the frequency of serotype 6C infections increasing from 3.3% to 17% between 2000 and 2005 and 2006–2008 (P = .02) [37].

### Table 3. Distribution of Penicillin-Nonsusceptible Serotype C Isolates Worldwide

<table>
<thead>
<tr>
<th>Location</th>
<th>No. of Strains</th>
<th>Source</th>
<th>Collection Year(s)</th>
<th>% Pcn-NS (No.)</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>North America</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>141 Blood</td>
<td></td>
<td>2006</td>
<td>32.6 (46)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>141 Blood</td>
<td></td>
<td>2007</td>
<td>31.2 (44)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60 Blood, respiratory, ear, sinus, NP</td>
<td>1989 (1); 1997–2009 (28)</td>
<td></td>
<td>46.7 (28)</td>
<td>Jacobs [18]</td>
</tr>
<tr>
<td></td>
<td>48 NP</td>
<td>1994–2007</td>
<td></td>
<td>22.2 (8)a</td>
<td>Nahm [33]</td>
</tr>
<tr>
<td>South America</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>19</td>
<td>CSF (16), NP (3)</td>
<td>1996–2007</td>
<td>5.3 (1)b</td>
<td>Campos [28]</td>
</tr>
<tr>
<td>Europe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portugal</td>
<td>106</td>
<td>NP</td>
<td>1996–2007</td>
<td>30.2 (32)</td>
<td>Nunes [34]</td>
</tr>
<tr>
<td>Ireland</td>
<td>2</td>
<td>Blood and/or CSF</td>
<td>2007</td>
<td>0</td>
<td>Vickers [35]</td>
</tr>
<tr>
<td>Spain</td>
<td>22 964</td>
<td>Blood, CSF, pleural space</td>
<td>1997–2009</td>
<td>77.0 (164)</td>
<td>Rolo [27]</td>
</tr>
<tr>
<td>Spain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Israel</td>
<td>106</td>
<td>Blood, ear, Conjunctiva, NP</td>
<td>1999–2008</td>
<td>5.7 (6)</td>
<td>Porat [26]</td>
</tr>
<tr>
<td>Africa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Africa</td>
<td>30</td>
<td>CSF (17), Blood (13)</td>
<td>2005–2006</td>
<td>0</td>
<td>du Plessis [29]</td>
</tr>
<tr>
<td>Asia</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>China</td>
<td>1662</td>
<td>NP, hypopharyngeal</td>
<td>1997–2008</td>
<td>0</td>
<td>Yao [36]</td>
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<tr>
<td>Hong Kong</td>
<td>730</td>
<td>NP</td>
<td>1999–2000; 2009–2010</td>
<td>55.9 (19)</td>
<td>Ho [31]</td>
</tr>
<tr>
<td>Australia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New South Wales</td>
<td>14</td>
<td>Blood, CSF, respiratory</td>
<td>2003 + unspecified years</td>
<td>7.0 (1)</td>
<td>Jin [19]</td>
</tr>
<tr>
<td>Australian Capital Territory</td>
<td>52</td>
<td>Invasive, respiratory</td>
<td>1999–2008</td>
<td>4.0 (2)</td>
<td>Zhuo [37]</td>
</tr>
</tbody>
</table>

Abbreviations: CSF, cerebrospinal fluid; NP, nasopharynx; NR, not reported; Pcn-NS, penicillin-nonsusceptible.

a Based on 36 serotype 6C and 12 serotype 6A isolates collected in 2007.

b Based on 16 isolates detected from cases with meningitis.
Akin to IPD, the frequency of NP colonization with serotype 6C isolates has been increasing since the introduction of the PCV7 vaccine. Although rates of colonization with PCV7 vaccine types decreased by 53.1% between 2001 and 2006 in Portugal, colonization with serotype 6C isolates increased dramatically during this same time period [39]. Among children residing in Massachusetts, the frequency of serotype 6C isolated from the NP increased from 0.6% (2 of 343) to 8.7% (36 of 416) between 1999–2000 and 2007, whereas the frequency of serotype 6A significantly decreased from 9.6% (33 of 343) to 2.9% (12 of 416) during the same time period [33]. Between 2008 and 2009, serotype 6C accounted for 11% of the pneumococcal isolates detected in the NP [40]. Although these data provide compelling indirect evidence that PCV7 does not provide protection against serotype 6C, the in vitro assays were of paramount importance in determining this.

**INABILITY OF PCV7 AND PPSV23 TO PROVIDE PROTECTION AGAINST SEROTYPE 6C DISEASE**

Both PCV7 and PPV23 vaccines include serotype 6B, but neither includes serotypes 6A or 6C. An in vitro opsonization assay to measure the protective effect of pneumococcal vaccines was used to determine whether PCV7 and PPV23 can protect against serogroup 6 disease. Among children who had received PCV7, high opsonic indices for serotypes 6A, 6B, and 6C were detected in 68%, 95%, and 26% of the children, respectively. Among adults who had received PPV23, high opsonic indices for serotypes 6A, 6B, and 6C were detected in 81%, 88%, and 52% of the adults, respectively [41]. Thus, high opsonic indices against serotype 6C were relatively infrequent among children and adults who had been immunized with PCV7 or PPV23. Specifically, the magnitude of the opsonic activity was 10–100-fold less for serotype 6C than for serotypes 6A and 6B. Taken together, the rise in serotype 6C disease after introduction of the PCV7 vaccine was likely due to the inability of PCV7 to induce robust levels of functional anti-6C antibodies in comparison with anti-6A and anti-6B antibodies.

**SEROTYPE 6D**

**Discovery, Serology, Chemical Structure**

Within the capsular locus, serotype 6A differs from serotype 6B because of an SNP in the wciP gene; serotype 6A differs from serotype 6C due to a variation in the wciN gene. Ostensibly, a serotype could exist that has the wciP gene of serotype B and the wciN gene of serotype C. This strain was generated in the laboratory and named TIGR6X1, or serotype 6D [42] (Figure 1).

Although extremely uncommon, invasive disease due to serotype 6D has been described in residents from Finland, Poland, Australia, and the United States [37, 43–45]. Serotype 6D has also been isolated from the sputum of a Canadian adult [46]. In contrast, serotype 6D strains have been commonly detected in the NP of children from Peru [47], Fiji [48], and Korea [49]. Moreover, serotype 6D strains account for approximately 10% of serogroup 6 strains associated with NP carriage in South Korea [50–52]. Given that pediatric pneumococcal carriage is a reservoir for adult IPD, continued surveillance is essential to determine whether IPD due to serotype 6D will increase in the future.

**Identification of Serotype 6D Strains**

The quellung reaction reagents, which were modified in 2009 by the Statens Serum Institut, can be used to presumptively identify a serotype 6D strain. The strain will be reactive with factor antisera 6c and 6d, but NOT factor antisera 6b*. In contrast, serotype 6A will only be reactive with factor serum 6b*; serotype 6B will only be reactive with factor sera 6c; and serotype 6C will only be reactive with factor sera 6d [53] (Table 2). Using molecular subtyping methods, a serotype 6D strain will have a shorter wciN gene (wciN6) that has been detected in serotype 6C strains [5, 18] and a wciP gene that has an SNP associated with serotype 6B strains [12].

**Evolution of Serotype 6D**

The serotype 6D capsular operon is highly conserved among serotype 6D strains, with approximately 99% homology between a Finnish and Korean strain [45]. An evolutionary tree of wciP, wzy, and wex concatenated gene sequences revealed that serotype 6D strains clustered within a clade of 6B strains [23]. Moreover, the serotype 6D capsular locus is 98.6% identical to a serotype 6C, with differences occurring in the noncoding regions and the wciP gene [23]. Therefore, it is possible that the serotype 6D capsule gene locus was initially generated by a recombination event between the capsular locus of a serotype 6B and serotype 6C strain.

The multilocus sequence typing data suggest that the serotype 6D capsular operon was inserted into strains from other serotypes, including those related to international clones. For example, among 24 serotype 6D isolates detected from the NP of Fijian children, 58.3% (14 of 24) were genetically related to serotype 6B-ST176, 37.5% (9 of 24) belonged to serotype 6A-ST473, and 4.1% (1 of 24) belonged to serotype 6C-ST4240 [54]. Moreover, in addition to the detection of novel ST [43, 45], serotype 6D strains that are genetically related to the Spain 23F-1 clone have been described [49].
**VACCINE PREVENTION OF SEROTYPE 6C AND 6D DISEASE WITH PCV13**

The 13-valent pneumococcal conjugate vaccine (PCV13), which was recently licensed in the United States [55], includes the PCV7 serotypes plus serotypes 1, 3, 5, 6A, 7F, and 19A. Thus, because PCV13 includes both serotypes 6A and 6B but neither serotypes 6C nor 6D, it is unknown whether this vaccine will provide protection against IPD due to either serotype 6C or serotype 6D; however, the in vitro data are quite promising.

Because serotype 6A has significant structural and immunologic similarity with serotype 6C, the inclusion of serotype 6A in PCV13 provides credence to the theory that PCV13 will provide protection against serotype 6C. Moreover, immunologic data indicate that the sera obtained from those immunized with PCV13 showed stronger opsonophagocytic assay (OPA) responses to serotype 6C than the sera obtained from those immunized with PCV7. Specifically, 96% of the samples from infants who had received a 3-dose series of PCV13 had positive serotype 6C OPA titer. In contrast, only 22% of the samples from infants who had received a 3-dose series of PCV7 had positive serotype 6C OPA titer [56]. Recent reports describing the early clinical experience with PCV13 suggest that it may reduce NP carriage due to serotype 6C [57]. Specifically, the NP carriage of serotype 6C was 3.7% among children who received at least 1 dose of PCV13, compared with 8.4% among children who received PCV7 (P < .01) [57]. It is still too early to tell whether PCV13 will also reduce the incidence of IPD due to serotype 6D; however, in 1 small study, the frequency of IPD due to PCV13 serotypes was markedly reduced among children who received this vaccine [58].

Preliminary in vitro data suggest that the serotype 6B component of PCV7 elicits antibodies that are cross-opsonic to serotype 6D (unpublished data). However, clinical data are not yet available to confirm these findings. Theoretically, evaluating the incidence of IPD due to serotype 6D before and after the introduction of PCV13 would indirectly determine whether this vaccine provides protection against serotype 6D. However, IPD due to 6D is rare. Thus, epidemiologic studies may need to focus on NP colonization in populations that frequently carry serotype 6D. Specifically, monitoring the frequency of NP carriage with serotype 6D in Korean children before and after the introduction of PCV13 might help determine whether PCV13 provides clinical protection against serotype 6D.

**CONCLUSION**

Although sustained reductions in invasive disease occurred after the licensure of PCV7 [2], considerable serotype replacement did occur [3]. For example, the newly discovered serotype 6C now accounts for most of the serogroup 6 IPD in the United States [5, 6]; fortunately, this significant rise has only had a minor impact on the overall incidence of IPD and has not eroded the efficacy of PCV7. Specifically, the overall incidence of IPD in 2007 was 45% lower than in 1998, and this dramatic decline was likely to due to the licensure of PCV7 in 2000 [2]. Moreover, based on immunologic data, IPD due to serotype 6C may significantly decrease with the introduction of PCV13 [55, 56]. Although serotype 6D rarely causes invasive disease, the preliminary in vitro data suggest that PCV13 will provide protection against disease due to serotype 6D, too.

Thus, at first blush, it appears that the incidence of IPD in countries that routinely vaccinate children with PCV13 will have further reductions in IPD and that the discovery of serotype 6C and 6D is a simple anomaly. However, in addition to the expansion of existing nonvaccine clones and the ability of vaccine-type clones to evade PCV13 by undergoing capsular recombination, a new method of serotype replacement has become apparent with the discovery of novel serotypes 6C and 6D—and more recently, serotypes 11E [59] and 20B [60]. Thus, the discovery of serotypes 6C and 6D may not have been an anomaly; rather, they may be a harbinger of the challenges of preventing pneumococcal disease in the serotype-specific conjugate vaccine era.

**Notes**

**Disclaimer.** University of Alabama Birmingham (UAB) has intellectual property rights on serotypes 6C and 6D, and M. H. N. is an employee of UAB.

**Financial support.** This work was supported by an NIH grant AI-31473.

**Potential conflicts of interest.** Both authors: No reported conflicts.

Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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